

**INTERNATIONAL SEARCH REPORT**

International application No.  
PCT/AU2004/000494

**A. CLASSIFICATION OF SUBJECT MATTER**

Int. Cl. <sup>7</sup>: C12N 15/29

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
SEE ELECTRONIC DATABASES BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
SEE ELECTRONIC DATABASES BELOW

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
DGENE, Genebank, EMBL, SwissProt, PIR, PubMed : SEQ ID 2, 4, 6, 8, 10, 12, 14, 16 ; Keywords: chalcone synthase, leucoanthocyanidin reductase, lcr, ban, dihydroflavonol 4-reductase, clover, trifolium.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No.     |
|-----------|---|---------------------------|
| P, X      | WO, A, 2003031622 (AGRICULTURE VICTORIA SERVICES PTY LTD ET.AL.) 17 April 2003 (SEQ ID 2 has 100% identity with Figure 143, SEQ ID 4 has 96% identity with Fig. 148, SEQ ID 6 has 93% identity with Figure 158, SEQ ID 8 has 94% identity with Figure 163, SEQ ID 10 has 100% identity with Figure 118, ) | 1-31                      |
| X         | WO, A, 2002057418 (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) 25 July 2002 (SEQ ID 2 has 92% identity with SEQ ID 1 on page 13)   | 1-3,6-8,10-17,19-23,25-29 |
| X         | SWISS-PROT database Accession Number P51088, Chalcone synthase 6. Howles, P. A. et al. 1 October 1996 (98% identity with SEQ ID 2)  | 1-3,6-8,10-17,19-23,25-29 |
| X         | SWISS-PROT database Accession Number P51083, Chalcone synthase 1. Arioli, T., et al. 1 October 1996 (97% identity with SEQ ID 2)  | 1-3,6-8,10-17,19-23,25-29 |

Further documents are listed in the continuation of Box C

See patent family annex

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| * Special categories of cited documents: |  |
| "A"                                      | document defining the general state of the art which is not considered to be of particular relevance   |
| "T"                                      | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  |
| "E"                                      | earlier application or patent but published on or after the international filing date  |
| "X"                                      | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone   |
| "L"                                      | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)  |
| "Y"                                      | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "O"                                      | document referring to an oral disclosure, use, exhibition or other means   |
| "&"                                      | document member of the same patent family  |
| "P"                                      | document published prior to the international filing date but later than the priority date claimed   |

Date of the actual completion of the international search  
28 June 2004

Date of mailing of the international search report

8 JUL 2004

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| C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |  |                               |
|---|--|-------------------------------|
| Category*   |  | Relevant to claim No.         |
| X   | GenPept database Accession Number CAA10131 Chalcone synthase, Dopico, B., et. al. 11 November 1998 (97% identity with SEQ ID 2)  | 1-3,6-8,10-17,19-23,25-29     |
| X   | SWISS-PROT database Accession Number Q9SEVO Leucoanthocyanidin reductase, Devic, M., et. al. 28 February 2003 (61% identity with SEQ ID 10)  | 1,2,4,6,7,9-16,18-22,24-28,30 |
| X   | GenPept database Accession Number AAF23859 11 January 2000 (61% identity with SEQ ID 10)   | 1,2,4,6,7,9-16,18-22,24-28,30 |
| X   | JEZ, J. M. et.al. Structure and Mechanism of Chalcone Synthase-like Polyketide Synthases, <i>Journal of Industrial Microbiology &amp; Biotechnology</i> (2001) 27:393-8  | 1-3,6-8,10-17,19-23,25-29     |
| X   | ARIOLI, T., et.al. In <i>Trifolium subterraneum</i> , chalcone synthase is encoded by a multigene family. <i>Gene</i> , 138:79-86 (1994) (SEQ ID 2 is 97% identity to seq. of Fig. 3)  | 1-3,6-8,10-17,19-23,25-29     |
| X   | CHARRIER, B., et.al. Molecular characterization and expression of alfalfa ( <i>Medicago sativa</i> L.) flavanone-3-hydroxylase and dihydroflavonol-4-reductase encoding genes. <i>Plant Molecular Biology</i> 1995 Nov;29(4):773-86 (See Figure 3) | 1,2,4,6,7,9-16,18-22,24-28,30 |
| X   | WO A1 2002066625 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION ET. AL.) 29 August 2002 (SEQ ID 12 and 14 and 16 have 72% identity to SEQ ID 28)  | 1,2,5,6,8-15,17-21,23-28,31   |
| X   | WO A1 2000022099 (GENESIS RESEARCH AND DEVELOPMENT CORPORATION LIMITED et. al.) 20 April 2000, (SEQ ID 12 and 14 have 56% identity to SEQ ID 323. SEQ ID 16 has 57% identity to SEQ ID 323)  | 1,2,5,6,8-15,17-21,23-28,31   |
| X   | EP A 1033405 (CERES INC.) 6 September 2000. (SEQ ID 4 has 77% identity with SEQ ID 66257 and SEQ ID 15839, SEQ ID 6 has 67% identity with SEQ ID 7420 and 7419)  | 1-3,6-8,10-17,19-23,25-29     |
| X   | WO A 2002010210 (BAYER AG) 7 February 2002. (SEQ ID 4 has 77% identity with SEQ ID 2451, SEQ ID 6 has 70% identity with SEQ ID 18.)  | 1-3,6-8,10-17,19-23,25-29     |
| X   | EP A 1254960 (UNILEVER PLC) 6 November 2002. (SEQ ID 8 has 81% identity with Fig.11.)  | 1-3,6-8,10-17,19-23,25-29     |
| P,X   | WO A 2003040306 (GENESIS RESEARCH AND & DEVELOPMENT CORPORATION LTD) 15 May 2003. (SEQ ID 8 has 78% identity with SEQ ID 119 and SEQ ID 186.)  | 1-3,6-8,10-17,19-23,25-29     |
| P,X   | WO A 2004020637 (INTERNATIONAL FLOWER DEVELOPMENTS PTY. LTD.) 19 March 2004. (SEQ ID 8 has 80% identity with disclosure on p.244-6.)   | 1-3,6-8,10-17,19-23,25-29     |
| X   | PIR database Accession Number T10231 Anther-specific protein homolog T11II1.90, Bevan, M., et. al. 16 July 1999. (77% identity with SEQ ID 4.)   | 1-3,6-8,10-17,19-23,25-29     |

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|--|---|---------------------------|
| Category*  | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No.     |
| X  | PIR database Accession Number T15054. Anther-specific protein - wood tobacco. Atanassov, I.I., et. al. 20 September 1999. (76% identity with SEQ ID 6.)   | 1-3,6-8,10-17,19-23,25-29 |
| X  | EMBL database Accession Number Y14507, Nicotiana sylvestris CHSLK gene. Atanassov I.I., et. al. 11 August 1997. (70% identity with SEQ ID 6.)   | 1-3,6-8,10-17,19-23,25-29 |
| X  | SWISS-PROT database Accession Number P51075, Chalcone synthase. Pellinen, R., et. al. 1 October 1996. (82% identity with SEQ ID 8.)   | 1-3,6-8,10-17,19-23,25-29 |
| X  | SWISS-PROT database Accession Number Q9FSB7, Chalcone synthase 3. Springob, K., et. al. (82% identity with SEQ ID 8), and, SPRINGOB, K., et. al. Specificities of functionally expressed chalcone and acridone synthases from <i>Ruta graveolens</i> , Eur. J. Biochem. 267(22):6552-9 (2000) | 1-3,6-8,10-17,19-23,25-29 |
| X  | TREMBL database Accesssion Number BAB84112. Chalcone synthase ( <i>Vitis vinifera</i> ). Goto-Yamamoto, N., et. al. 19 July 2002. (82% identity with SEQ ID 8.)   | 1-3,6-8,10-17,19-23,25-29 |

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**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
  - a. type of material
    - a sequence listing
    - table(s) related to the sequence listing
  - b. format of material
    - in written format
    - in computer readable form
  - c. time of filing/furnishing
    - contained in the international application as filed
    - filed together with the international application in computer readable form
    - furnished subsequently to this Authority for the purposes of search
2.  In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

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**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

The Applicant has claimed more than one invention. Rule 13.1 of the PCT states the principle that an International Application should relate to only one invention or, if there is more than one invention, that the inclusion of those inventions in one International Application is only permitted if all inventions are so linked as to form a single general inventive concept.

*(continued on Extra Sheet)*

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.

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**Supplemental Box**

(To be used when the space in any of Boxes I to VIII is not sufficient)

**Continuation of Box No: III**

Rule 13.2 of the PCT defines the method for determining whether the requirement of unity of invention is satisfied in respect of a group of inventions claimed in an International application. Unity of invention exists only when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding "special technical features." The expression "special technical features" is defined in Rule 13.2 as meaning those technical features that define a contribution which each of the inventions, considered as a whole, makes over the prior art. The determination is made on the contents of the claims as interpreted in light of the description and drawings (if any).

**Invention 1** : Polypeptide and polynucleotide encoding chalcone synthase of the formula TrCHSa3, represented by SEQ ID NOS 1 and 2. This invention is recited in claims 1 - 3, 6 - 8, 10 - 17, 19 - 23, 25 - 29 (in part).

**Invention 2** : Polypeptide and polynucleotide encoding chalcone synthase of the formula TrCHSc, represented by SEQ ID NOS 3 and 4. This invention is recited in claims 1 - 3, 6 - 8, 10 - 17, 19 - 23, 25 - 29 (in part).

**Invention 3** : Polypeptide and polynucleotide encoding chalcone synthase of the formula TrCHSf, represented by SEQ ID NOS 5 and 6. This invention is recited in claims 1 - 3, 6 - 8, 10 - 17, 19 - 23, 25 - 29 (in part).

**Invention 4** : Polypeptide and polynucleotide encoding chalcone synthase of the formula TrCHSh, represented by SEQ ID NOS 7 and 8. This invention is recited in claims 1 - 3, 6 - 8, 10 - 17, 19 - 23, 25 - 29 (in part).

**Invention 5** : Polypeptide and polynucleotide encoding dihydroflavonol 4-reductase of the formula TrBANA, represented by SEQ ID NOS 9 and 10. This invention is recited in claims 1, 2, 4, 6 - 7, 9, 10 - 16, 18 - 22, 24 - 30. (in part)

**Invention 6** : Polypeptide and polynucleotide encoding leucoanthocyanine reductase of the formula TrLARA, represented by SEQ ID NOS 11 and 12. This invention is recited in claims 1, 2, 5, 6, 8 - 15, 17 - 21, 23 - 26, 28, 29, 30. (in part).

**Invention 7** : Polypeptide and polynucleotide encoding leucoanthocyanine reductase of the formula TrLARB, represented by SEQ ID NOS 13 and 14. This invention is recited in claims 1, 2, 5, 6, 8 - 15, 17 - 21, 23 - 26, 28, 29, 30. (in part).

**Invention 8** : Polypeptide and polynucleotide encoding leucoanthocyanine reductase of the formula TrLARC, represented by SEQ ID NOS 15 and 16. This invention is recited in claims 1, 2, 5, 6, 8 - 15, 17 - 21, 23 - 26, 28, 29, 30. (in part).

Each of the above enzymes may be involved in flavonoid biosynthesis in plants, and more specifically the modification of the content of condensed tannins, but this not novel, as noted in the following abstract. Therefore this cannot be used as a special technical feature providing unity to all of the sequences.

XIE DY et. al. Science (Jan 2003)299(5605):396-9 Role of anthocyanin reductase, encoded by BANYULS in plant flavonoid biosynthesis.

Although some of the claims share a technical feature whereby they are a chalcone synthase, a dihydroflavonol 4-reductase or a leucoanthocyanine reductase, these are not special technical features because none of these are novel enzymes. For example, the following three journal abstracts indicate that none of these enzymes are novel, and therefore cannot be used as a special technical feature uniting the inventions into three groups.

NAPOLI C, et. al. The Plant Cell (1990) 2:279-89 Introduction of a Chimeric Chalcone Synthase Gene into Petunia Results in Reversible Co-suppression of Homologous Genes *in trans*.

JOHNSON ET, et. al. Plant Journal (2001) 25(3):325-33 Alteration of a single amino acid changes the substrate specificity of dihydroflavonol 4-reductase.

TANNER GJ et.al. Anal Biochem 1993 March 209(2):274-7 Synthesis of 3,4-cis-[3H]leucocyanidin and enzymatic reduction to catechin

(Continued on Extra Sheet.)

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**Supplemental Box**

(To be used when the space in any of Boxes I to VIII is not sufficient)

**Continuation of Box No:**

Furthermore, given the nature of the claims and the invention, it is appropriate to apply the Markush approach. Using the Markush approach to analyse the unity of inventions, although the 8 gene transcripts all have the common property (A) that they are involved in flavonoid biosynthesis in plants, and more specifically the modification of the content of condensed tannins, (B)(1) there is no common structure present in all of the genes; and (B)(2) there is no single recognised class or group of compounds embracing all the genes claimed. It is contrary to normal classification to group together such diverse genes. Thus according to Markush, it is appropriate to classify the genes in terms of the 8 individual groups and thus these groups represent 8 different inventions.

In order to search each of the inventions, this could only be done by consideration of each of the sequences, thereby requiring eight separate searches. As a service to the applicant, this office would for the purposes of this search, consider Inventions 1 and 5 together for a single search fee. Each of the other sequences will be considered as single inventions and each attracts a search fee.

The applicant chose to pay the six extra fees being sufficient to search and examine all eight inventions.